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Antioxidant activity of a new diarylheptanoid from *Zingiberofficinale*

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ABSTRACT - Rhizomes of *Zingiber officinale* commonly known as ginger are used popularly as spices. This drug is also used in traditional system of medicine as antiulcer and antioxidant. Many phytoconstituents had been isolated from oil and oleoresins of ginger. In the present study, a new diarylheptanoid was isolated from the spent ginger devoid of oleoresin. The spent ginger was extracted with 50% methanol and partitioned with butanol. Column chromatography and preparative HPLC of the butanolic extract resulted in isolation of a new compound which was characterized as 3, 5 diacetoxy-7-(3, 4 dihydroxy phenyl)-1-(3, 4 dihydroxy phenyl) heptane (ZA6). *In vitro* DPPH, superoxide scavenging bioassay indicated that, this diarylheptanoid is a potent antioxidant.
KEY WORDS - *Zingiber officinale*, diarylheptanoids, DPPH assay, superoxide scavenging.

INTRODUCTION

Zingiber officinale Roscoe belonging to family Zingiberacea has been used as a spice and medicine for thousands of years (1). Utilization of plants and other crude preparations for therapeutic reason have several drawbacks including lack of marker compound to maintain uniformity of the herbal preparation. The isolation and characterization of marker compounds is one of the most important areas of research in medicinal plants. The isolation of markers permits structural determination of bioactive compounds that may enable production of synthetic material, incorporation of structural modification and rationalization of mechanism of action (2).

Many ayurvedic formulations like "trikatu" are known to contain ginger extract as one of the ingredient. Gingerol and shogaol isolated from oleoresin are the only reported markers of ginger. Hence, in present study an attempt was made to isolate a new chemical constituent of *Zingiber officinale* from spent ginger extract using column chromatography and preparative HPLC and evaluate its anti-oxidant potential

MATERIALS AND METHODS

Raw material of fresh ginger was collected and authenticated at Natural Remedies Pvt. Ltd. The raw material was subjected to supercritical fluid extraction (SFE) to collect oleoresin devoid spent. The spent ginger obtained after SFE was extracted with 50% methanol in water for 4hrs. at 70^0C. The extract was partitioned with butanol and dried in vacuum tray drier at 70 ^{0}C. The butanolic extract was fractionated using silica gel and pet-ether, ethyl acetate, methanol in varying concentration as stationary and mobile phase. The TLC of butanolic extracts and fractions of the column was optimized using precoated 60F254 silica plates with toluene: butanol: glacial acetic acid: water - 6:4:5:1 and pet-ether: chloroform: ethyl acetate: acetone - 2:5:1:2 with anisaldehyde as detecting agent. The fraction obtained from butanolic extract at 50-75% ethyl acetate in pet-ether was selected and refractionate using another silca column with pet-ether:chloroform:acetone:methanol in different concentration. The fraction obtained at 12.5-15% acetone in chloroform was collected and purified by preparative HPLC (shimadzu consisting of LC10ATVP pump, reodyne injector and class VP-6 software) as a new diarylheptanoid. The RP merk-C-18 (cromosil, 250x20mm, 5µ stationary phase) column with isocratic elution and 40% acetonitrile in water was used for purification of an isolated diarylheptanoid. The volume injected was 20ml of an isolated compound dissolved in methanol (HPLC grade). The detection was carried out at 205-330nm using photodiode array detector.

An isolated compound was characterized by NMR (^{1}H and ^{13}C). An isolated compound was subjected to three different bioassay namely DPPH inhibition, superoxide scavenging and elastase inhibition for screening its antioxidant and antiulcer activity.

RESULTS

An isolated diarylheptanoid was purified by preparative HPLC and characterized using TLC and NMR.

Analytical HPLC chromatogram of isolated compound ZA6.

Peak no.	Retention time	Area of peak	Concentration.
1	6.375	1334137	3.8249
2	13.420	71634	0.2054
3	14.068	745251	2.1366
4	15.182	596840	1.7111
5	15.770	1815950	5.2063
6	17.031	1116482	3.2009
7	17.641	1185774	3.3996
8	18.185	28013814	80.3151
Total		34879880	100.00

Preparative HPLC chromatogram of isolated compound ZA6

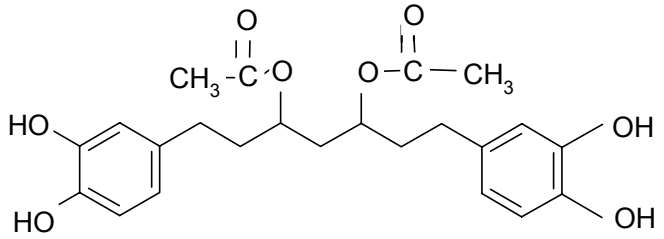
Peak no.	Retention time	Area of peak	Concentration.
1	3.883	200694	0.1356
2	4.117	81728	0.0552
3	4.537	141506	0.0956
4	7.806	806391	0.5449
5	7.957	355987	0.2406
6	9.353	145772897	98.5112
7	12.107	5493	0.0037
8	13.270	91293	0.0617
9	13.512	519937	0.3514
Total		147975924	100.00

^{1}H NMR	
δ1.877-2.026	Singlet-CH_3 of ester
δ6.511-6.735	Aromatic region
δ3.844-3.859	Doubletof heptane chain.

^{13}C NMR	
δ 47.88-49.16	Heptane
δ115.12-115.24	CH_3 of ester
δ119.58	C-OH
δ133.13	Aromatic carbons
δ142.70	Aromatic carbons attached to heptane
δ144.49	C=O

The physical characters of isolated diarylheptanoid were found to be sticky having melting point 53 ^{0}C. The structure was predicted as 3,5-diacetoxy-7-(3,5 dihydroxy phenyl)-1-(3,4 dihydroxy phenyl)heptane on the basis of NMR(^{1}H and ^{13}C).

Structure of an isolated new diarylheptanoid is predicted as:



3,5 diacetoxy-7-(3,5 dihydroxy phenyl)-1-(3,4 dihydroxy phenyl)heptane.

Bioassay: *Isolated diarylheptanoid was tested for its in vitro superoxide scavenging activity using PMS-NADH system(3), antioxidant activity DPPH system(4) and antiulcer activity using elastase inhibition activity(5). It showed to posses potent antioxidant and antielastase activities.*

Percentage inhibition using different concentration of a new diarylheptanoid with standard

Sample	Concentration	% inhibition	Activity
Diarylheptanoid	10µg/ml	18.66	Superoxide scavenging
	50µg/ml	35.43	
Gallic acid (std.)	10µg/ml	30.90	
	50µg/ml	54.81	
Diarylheptanoid	10µg/ml	15.55	antioxidant
	50µg/ml	76.01	
Gallic acid (std.)	1.5µg/ml	14.56	
	5µg/ml	66.69	
Diarylheptanoid	5µg/ml	-	antielastase
	50µg/ml	1.97	
Ursolic acid (std.)	5µg/ml	26.34	
	20µg/ml	55.92	

DISCUSSION

A 50% methanolic extract was prepared and standardized for pilot scale extraction. Methanolic extract of ginger has been reported for different pharmacological activities like antioxidant, antiviral and antielastase activity etc (6-7). An attempt was made to made to fractionate the aqueous methanolic extracts to isolate the phytoconstituents. TLC is one of the most commonly used procedures to select the phytoconstituents.

Isolation and purification of chemical constituents

Isolation of chemical constituents was done by fractionation using repeated column chromatography. The butanolic extract obtained from 50% methanolic extract was subjected to column chromatography using 17 different columns and 94 fractions ranging from 4-2 lts volume were collected. The fractions were rechromatographed on silica gel G and diaion HP-20 columns to get the isolated compound ZA6. Analytical HPLC method was developed at Natural Remedies (Bangalore) using HPLC (Shimadzu) with RP C-18 column and acetonitrile in d.w (30-65%) as mobile phase and PDA and ELSD detector. The detection was carried out at 205-330nm.

The purification of the isolated compounds was done by using preparative HPLC which is a popular tool for isolation of phytoconstituents in industries. A gradient elution RP-HPLC separation of isolated compounds has been developed which gives better separation than isocratic separation (8). Semi preparative RP-HPLC was used as the first separation technique to isolate the pure compounds (9).

Characterization of isolated compounds

Isolation and purification of compounds by column chromatography and preparative HPLC (9, 10). The ^{1}H-NMR and ^{13}C-NMR spectrums was used to characterize the isolated compounds (11). Isolation of different diarylheptanoids has been reported from different extracts of ginger (12-14). The structure of the isolated compound ZA6 in our study shows 3, 5 diacetoxy-7-[3, 4 dihydroxy phenyl]-1-[3, 4 dihyroxy phenyl] heptane.

Evaluation of bioactivity of isolated compounds

As ginger is widely used as stomachic (15), antiulcer (16) and antioxidant (10) and the diarylheptanoids are also reported as antiulcer (15), antifungal (17), prostaglandin inhibitor (18), antihepatotoxic (19), cytotoxic and apoptotic (20). The isolated compounds were tested for antiulcer and antioxidant activity by using invitro methods. The three bioassays namely DPPH inhibition (4), superoxide scavenging (3) and elastase inhibition (5) of isolated compound ZA6 was performed to determine antioxidant and antiulcer property.

CONCLUSION

Authenticated samples of rhizomes of *Zingiber officinale* Roscoe belonging to family Zingiberacea were selected for isolation of phytoconstituents.

Spent ginger devoid of oleoresin was collected and extracted with water, methanol and aqueous methanol (50%) in different lots to select an extract with maximum number of phytoconstituents.

A TLC system with toluene: butanol: glacial acetic acid: water (6:4:5:1 as mobile phase and anisaldehyde as detecting agent) was optimized to identify the

phytoconstituents. A 50% methanolic extract of spent ginger was found to contain maximum number of phytoconstituents and hence it was selected for isolation of markers. The methanolic extract was partitioned with butanol and the butanolic extract was fractionated by repeated column chromatography using silica gel and diaion HP-20 as stationary phase and the solvents with varying polarity as mobile phase. This method was found to be suitable for fractionation/isolation of phytoconstituents at commercial scale. A new method of HPLC analysis was developed and standardized for identification of the isolated compounds. On the basis of purity and yield one isolated compound was selected for further purification by preparative HPLC.

An isolated compound was characterized as 3,5-diacetoxy-7-[3,4,dihydroxyphenyl]-1-[3,4, dihyroxy-phenyl] heptane (ZA6).

In vitro antioxidant screening showed that, the isolated compound ZA6 was found to posses antioxidant activity with 15.55 and 76.01% inhibition and superoxide scavenging activity with 18.66 and 35.43% inhibition at 10 and 50µg/ml concentration whereas, i*n vitro* antielastase screening showed that, the isolated compound ZA6 was found to posses antielastase activity with 1.97% inhibition at 50µg/ml concentration.

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